Influence of amino acids and branch chain organic acids on ruminant fiber fermentation
by Connie Kay Clark

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Science
Montana State University

Abstract:
Amino acids and branched chain organic acids were examined for possible positive effects on fiber fermentation in the rumen. Nine L-isomer amino acids and three branch chain organic acids were added as single amendments to in vitro buffer to evaluate influence of these additions on fermentation kinetics of native winter range forage collected via esophageal fistula. Amino acids provided 50 mg nitrogen per 100 ml buffer and branch chain organic acids were added at a .01% level. Dry matter (DM) and neutral detergent fiber (NDF) disappearance at 48 h (41.2% and 53.1%) were similar (P>.05) for buffer amendments and the control, although treatments differed significantly (P<.05). In comparison to the control, methionine, arginine, and cysteine increased (PC.05) both DM and NDF fermentation rate, and histidine and leucine influenced only DM fermentation rate. The correlation between DM fermentation rate and extent was positive and significant (P<.01). Lag time calculated for DM (29.1 h) and NDF (23.3 h) was similar for all treatments.

Methionine was further evaluated in an in situ trial. Three isonitrogenous isocaloric (.32 kg crude protein and 1.23 kg TDN) supplements, DL-methionine-urea, soybean meal (SBM), and urea, were formulated which contained: 4, 0, 63, 33; 0, 81, 0, 19; and 0, 0, 67, 33% nitrogen from DL-methionine, SBM, urea and beet pulp. Cannulated cows receiving the supplements were maintained on a mixture of 75% mature grass hay and 25% barley straw. DL-methionine-urea supplementation increased (P<.05) DM fermentation rate in comparison to the other supplements. Estimated rumen digestibility (48%) was improved (PC.01) and particulate passage rate (4.2 %/h) was slower (PC.05) with DL-methionine-urea and urea supplementation as compared to SBM. Neutral detergent fiber fermentation followed similar trends; however, no significant differences could be detected. Lag times (1.5 h) were calculated for NDF only.

A heifer growth trial was conducted utilizing the three supplements evaluated in the in situ trial and a SBM-urea supplement. Heifers were fed the same low quality roughage as described for the in situ trial. No differences were detected for average daily gain, intake, or feed efficiency.

INFLUENCE OF AMINO ACIDS AND BRANCH CHAIN ORGANIC ACIDS ON RUMINANT FIBER FERMENTATION

by

Connie Kay Clark

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Animal Science

MONTANA STATE UNIVERSITY
Bozeman, Montana

May, 1986

APPROVAL

of a thesis submitted by

Connie Kay Clark

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

5/14/86
Date

Mark K Peterson
Chairperson, Graduate Committee

Approval for the Major Department

May 14, 1986
Date

Arthur E. Linton
Head, Major Department

Approved for the College of Graduate Studies

May 20, 1986
Date

Henry L Parsons
Graduate Dean

STATEMENT OF PERMISSION TO COPY

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University, I agree that the Library shall make it available to borrowers under the rules of the Library. Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgement of source is made.

Permission for extensive quotation from or reproduction of this thesis may be granted by my major professor, or in his absence, by the Director of Libraries when, in the opinion of either, the proposed use of the material is for scholarly purposes. Any copying or use of the material in this thesis for financial gain shall not be allowed without my written permission.

Signature Connie Kay Clark

Date May 14, 1986

ACKNOWLEDGMENTS

I wish to express sincere appreciation to the following people:

Mark Petersen for his guidance, assistance and support in all areas of my graduate program;

Kris Havstad, Walt Newman, Rodney Kott and Ray Ansotegui for serving as members of my graduate committee;

Nancy Roth and Meg LaVigne for assistance with laboratory analysis;

Jim Fadal and Mike McInerny for their knowledge and help with the statistical analysis and;

Jeanne Blee for her guidance with typing the thesis.

I would like to extend a special thanks to my husband, Mike, and our family for their understanding and support. I dedicate this thesis to my late grandpa, Lawrence H. Swenson, from whom I developed an interest in agriculture.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Amino acids and branched chain organic acids were examined for possible positive effects on fiber fermentation in the rumen. Nine L-isomer amino acids and three branch chain organic acids were added as single amendments to *in vitro* buffer to evaluate influence of these additions on fermentation kinetics of native winter range forage collected via esophageal fistula. Amino acids provided 50 mg nitrogen per 100 ml buffer and branch chain organic acids were added at a .01% level. Dry matter (DM) and neutral detergent fiber (NDF) disappearance at 48 h (41.2% and 53.1%) were similar ($P>.05$) for buffer amendments and the control, although treatments differed significantly ($P<.05$). In comparison to the control, methionine, arginine, and cysteine increased ($P<.05$) both DM and NDF fermentation rate, and histidine and leucine influenced only DM fermentation rate. The correlation between DM fermentation rate and extent was positive and significant ($P<.01$). Lag time calculated for DM (29.1 h) and NDF (23.3 h) was similar for all treatments.

Methionine was further evaluated in an in situ trial. Three isonitrogenous isocaloric (.32 kg crude protein and 1.23 kg TDN) supplements, DL-methionine-urea, soybean meal (SBM), and urea, were formulated which contained: 4, 0, 63, 33; 0, 81, 0, 19; and 0, 0, 67, 33% nitrogen from DL-methionine, SBM, urea and beet pulp. Cannulated cows receiving the supplements were maintained on a mixture of 75% mature grass hay and 25% barley straw. DL-methionine-urea supplementation increased ($P<.05$) DM fermentation rate in comparison to the other supplements. Estimated rumen digestibility (48%) was improved ($P<.01$) and particulate passage rate (4.2 %/h) was slower ($P<.05$) with DL-methionine-urea and urea supplementation as compared to SBM. Neutral detergent fiber fermentation followed similar trends; however, no significant differences could be detected. Lag times (1.5 h) were calculated for NDF only.

A heifer growth trial was conducted utilizing the three supplements evaluated in the in situ trial and a SBM-urea supplement. Heifers were fed the same low quality roughage as described for the in situ trial. No differences were detected for average daily gain, intake, or feed efficiency.

Chapter 1

INTRODUCTION

Agriculture is challenged to feed an ever-increasing population with declining available resources. The ruminant provides man with a vital link to an abundant store of organic energy, forage, which supplies a source of nutrients for the ruminant that cannot be used by man. Fiber is a resource found in many areas unsuitable for cultivation or human residence. Equipped with a unique fermentation vat, ruminants are capable of utilizing fiber and complement man's need for energy and high quality protein without direct competition with man for food or land.

Forage harvesting by the ruminant animal may be the sole means of utilizing the rangelands in Montana. Although range plants can provide an adequate diet for the ruminant, the nutritional status of range plants vary substantially depending on the season of the year. Fiber digestibility may range from sixty-seven percent in June down to forty-nine percent or lower in December. Winter range supplementation with a protein source has been a common management practice to help maintain animals on winter range. A supplement program capable of increasing fiber digestibility and improving diet utilization by the animal could benefit the producer. In viewing the price-cost squeeze experienced by animal industries, a decrease in supplemental feed cost as well as better diet utilization by animals, may improve profits for producers.

In order to approach optimum rumen fermentation of winter range forage, minimum ruminal conditions are required. The rumen provides a suitable environment for anaerobic microorganisms to ferment fiber the animal consumed, and subsequently produce volatile fatty acids which are used by the animal as energy. The microbial population of the rumen may have specific nutritional requirements to obtain an optimum level of growth and reproduction which could enhance fiber fermentation. The importance of meeting nutritional needs of microbes may be magnified for ruminants on winter range as the nutrient supply for rumen microorganisms fermenting low quality forage could put limiting restraints on microbial growth and reproduction.

Cellulolytic bacteria are necessary for fiber digestion, but other bacteria must not be ignored because many of the microorganisms work in a synergistic fashion to accomplish fermentation. Providing rumen microorganisms with readily accessible amino acids or branch chain organic acids may enhance turnover of the microbial population. Although cellulolytic bacteria utilize ammonia as a major source of nitrogen, small amounts of amino acids or branch chain organic acids added to rumen bacteria are known to stimulate growth. It has been demonstrated that amino acids and branch chain organic acids improved microbial growth rate, but it is not known how this may influence fermentation.

This study had three objectives:

1) evaluate influence of amino acids and branch chain organic acids on fermentation of winter range forage,

2) incorporate an amino acid or branch chain organic acid into a supplement for cows consuming a low quality

roughage and evaluate influence of the supplement on fermentation parameters, and

3) evaluate influence of the supplement onperformance of growing heifers.

The study consisted of three phases. The first phase investigated the influence of single amino acid or branch chain organic acid addition to in vitro buffer solution on fermentation of native winter range forage, and served as a screening procedure for the following phases. An in situ trial was completed for phase two, in which a DL-methionine-urea supplement was compared to soybean meal and urea supplements. The final phase evaluated feedlot performance of heifers receiving a low quality forage supplemented with one of four supplements; DL-methionine-urea, urea, soybean meal, or soybean meal-urea.

Chapter 2

LITERATURE REVIEW

The ruminant is unique in its ability to harvest highly fibrous plants and produce nutritious food and products for man. To accomplish fiber utilization, the ruminant animal and rumen microbe populations live in a symbiotic relationship. With ingestion of feed, the ruminant provides a nutrient source for the microorganisms to grow and reproduce. The ruminant also contributes a suitable microbial environment with optimum temperature, pH, and mixing of rumen contents.

The rumen microorganisms provide energy substrate, protein and vitamins for the ruminant animal. An important function of the microbes is the production of cellulase and hemicellulase (Smith et al., 1973; Dehority, 1968), enzymes which enzymatically degrade fibrous digesta. Cellulose and hemicellulose supply an abundant source of carbon and energy in the ruminant's diet and without the fermentation action of the rumen bacteria these nutrients would be unavailable to the animal. The microbe population utilizes the carbon and energy of cellulose to produce volatile fatty acids such as acetic, propionic and butyric acid which the animal absorbs into the blood and uses for energy.

The microbial biomass of the rumen is composed of a multitude of differing microbe species capable of many biochemical functions (Hungate, 1966). Rumen bacteria appear to be more essential than

protozoa because rumen fermentation can proceed normally in defaunated animals (Eadie, 1962). Bacteria can be roughly divided into three groups according to their activity: cellulolytic bacteria, amylolytic bacteria and proteolytic bacteria, however many of the activities of individual species overlap with other species and it is difficult to designate bacteria into specific groups. Important cellulolytic species include Ruminococcus albus, Ruminococcus flavefaciens, Bacteroides succinogens, and Butyrivibrio fibrisolvens (Wolin, 1979).

To understand the process of cellulose fermentation, all species of rumen microbes need to be considered as fermentation occurs through the interaction of many species. Only a few of the predominant species produce cellulase for degradation. The hydrolysis products released from cellulose degradation are utilized as fermentation substrates by noncellulolytic as well as cellulolytic microorganisms. This reduces end product inhibition which improves the efficiency of enzymatic substrate degradation.

Bacterial Nitrogen and Branch Chain Organic Acid Requirements

Bryant (1973) reviewed nutrient requirements of cellulolytic bacteria. Three of the major cellulolytic species, Bacteroides succinogens, Ruminococcus albus, and Ruminococcus flavefaciens, required ammonia as the essential nitrogen source during growth regardless of the amount of amino acid and peptide nitrogen in the medium (Bryant and Robinson, 1962). Some strains are incapable of utilizing organic nitrogen source. These bacteria possibly lack the mechanisms to transport most amino acids or peptides into the cell.

Studies with Butyrivibrio fibrisolvens, a cellulolytic species, have indicated amino acids are essential for growth in some strains (Bryant and Robinson, 1962; Gill and King, 1958).

Branch chain organic acids, isovaleric, isobutyric and 2-methylbutyric, are required by major cellulolytic species (Bryant and Robinson, 1962). Specific requirements for branched chain organic acids of cellulolytic bacteria has been studied (Dehority et al., 1967; Allison et al., 1962a). Bacteroides succinogens require a combination of either isobutyric acid or 2-methylbutyric acid with valeric acid (straight chain fatty acid). Ruminococcus albus exhibited a requirement for isobutyrate or 2-methylbutyerate to achieve maximum growth. The Ruminococcus albus bacteria incorporated the branched chain organic acids into branched chain 14- and 16-carbon fatty acids or 14- and 16-carbon aldehydes (Allison et al., 1962b). Ruminococcus flavefaciens can utilize isobutyric, isovaleric, or 2-methylbutyric acid to maximize growth. Isovaleric acid was found to serve as a carbon skeleton for leucine synthesis by R. flavefaciens and isobutyric acid served the same function for valine synthesis (Allison et al., 1962a). Ruminococcus flavefaciens have limited ability to incorporate exogenous branched chain amino acids. Allison (1969) later reported that in addition to synthesis of valine, leucine, long chain branched fatty acids and aldehydes, the bacteria utilize shorter branched chain organic acids to synthesize isoleucine.

There is evidence of nutritional interdependence among rumen bacteria. Miura, et al. (1980) reported growth stimulation of Bacteroides amylophilus by starch resulted in successive growth of

amino acid-dependent Megasphaera elsdenii and branch chain fatty acid-dependent Ruminococcus albus in a media lacking both amino acids and branch chain fatty acids, indicating interdependence among the three bacteria species. Allison (1978) has also reported branch chain organic acid production by Megasphaera elsdenii. Further investigation by Muira, et al. (1983) demonstrated mixed rumen bacteria interact nutritionally in the same manner as revealed in pure culture mixture. The microbial population changes in relation to alterations of amino acid and branch chain fatty acid concentrations. Preformed amino acids or branch chain fatty acids are not essential for growth of branch chain fatty acid-dependent bacteria (Slyter and Weaver, 1971); however lag in the appearance of cellulose digestion may reflect time required for producing branch chain fatty acids via nutritional interdependence.

Bacterial Growth Rate

Currently in the literature, several publications report responses in rumen bacterial growth rate with the addition of amino acids, however; not much is available on the influence of amino acid additions on fermentation rate in the rumen. There are several factors in the complex ecosystem of the rumen that can have an effect on growth rate of microorganisms. Rates of hydrolysis of polymeric carbon and nitrogen sources, concentrations of permeable macronutrients used by microorganisms, concentrations of potentially growth-limiting micronutrients, inhibitory agents, and pH may be influential factors on growth rate (Wolin, 1979). The effect of

supplying readily available amino acids to rumen microorganisms may have an impact on microbial growth rate. An estimation that 60 to 80% of microbial nitrogen was derived from ammonia and 40 to 60% from amino acid- and peptide-nitrogen has been reported (Al-Rabbat et al., 1971; Nolan and Leng, 1972). An optimum ratio, 75% nonprotein nitrogen to 25% amino acid nitrogen, for rumen microbial growth has been indicated (Maeng et al., 1976).

A study conducted by Maeng and Baldwin (1976a) revealed that the addition of small amounts of amino acids to a diet containing urea as the nitrogen source improved rumen microbial yield. The greatest impact upon microbial yields with amino acid additions occured immediately after feeding and became less evident as time passed. The microbial nitrogen, protein and cell yield per mol ATP and per mol VFA was increased ($P<.01$) by amino acid containing treatments. The projected microbial nitrogen, protein, and cell yields per day and per kg of diet dry matter also was significantly improved with the addition of amino acids. A second study completed by Maeng and Baldwin (1976b) indicated a stimulatory microbial response with partial substitution of amino acids for urea. Amino acids replaced 25% of urea-nitrogen in a growth media. Growth rate of rumen bacteria was twofold and mean doubling time was reduced by half.

The relative degree to which ATP or other energy-rich compounds produced from catabolic activities are utilized by anabolic activities of the cell is referred to as energetic uncoupling. To obtain maximum microbial cell yields per unit of nutrient input, the rate of ATP production from fermentation reactions must equal the usage rate by

biosynthetic reactions at all times or in other words, the energetic uncoupling must be minimized. Hespell and Bryant (1979) suggest the stimulated bacterial growth response observed with amino acid additions is due to a decrease in the degree of energetic uncoupling with the rumen microbial population as a whole.

Studies have indicated the addition of branched chain organic acids have a positive influence on bacterial growth in the rumen. This is especially true of cellulolytics which have been shown to have nutrional requirements for branched chain organic acids. Russell and Sniffen (1984) reported the addition of low concentrations of isovaleric and 2-methylbutyric acid improved synthesis efficiency of rumen bacterial protein from carbohydrates in vitro. Bacterial growth measured by cell protein synthesis was increased 11.2% and 16.4% with the addition of isovaleric and 2-methylbutyric acid. A combination of isovaleric, isobutyric, 2-methylbutyric, and valeric acids produced a 18.7% increase in protein synthesis when added to in vitro inoculum. Branched chain organic acids have also increased bacterial growth measured in the rumen of sheep consuming a low quality forage supplemented with urea, isovalerate, isobutyerate, and 2-methylbutyerate (Hemsley and Moir, 1963).

Branched chain organic acids have been shown to effect digestion of forage. Isovaleric and isobutyric acid were added to an in vitro system fermenting purified cellulose with urea serving as the nitrogen source. Digestibility values at 30 hours revealed a substantial increase for the branched chain fatty acid treatment in comparison to the control (Bentley et al., 1955). Soofi et al. (1982) more recently

reported similar results. The addition of branched chain volatile acids increased ($P<.05$) in vitro dry matter digestibility at 48 hours, 48.1% digested verses 45.3% digested in the control. Gorosito et al. (1985) also studied in vitro digestion of forage as influenced by additions of isovaleric, isobutyric, 2-methylbutyric and valeric acid. All acids except valeric had positive effects on fermentation as indicated by increased cell wall digestibility at 24 hours and improved ammonia utilization. The greatest improvement for cell wall digestion was noted for the grass hay although alfalfa hay cell wall digestibility was also increased.

Hemsley and Moir (1963) reported an increase in intake and passage rate when isobutyric, isovaleric and 2-methylbutyric acid were added to a urea supplement provided to sheep consuming a low quality forage. Felix et al. (1980) fed isoacids with urea to lactating cows and indicated no influence on nitrogen or dry matter digestibility; however loss of urinary nitrogen was decreased and percent of absorbed nitrogen retained was higher indicating an improvement in utilization of nitrogen.

Fiber Fermentation

Forage digestion in the rumen involves complex relationships and interactions among the animal, microbial populations in the rumen, and plant components. Understanding ruminal digestion is simplified when the process is divided into four components: digestion rate, digestion lag, extent of digestibility, and passage rate (Mertens, 1977).

The first component to be addressed is digestion rate. Rate of digestion has been suggested as being important in assessing forage quality and voluntary intake (Crampton, 1957; Thorton and Minson, 1972). The theory is that enhancing rate of digestion would increase disappearance of fiber from the rumen; therefore, freeing space for additional intake. Mertens and Ely (1979) reported simulation results in which values for coastal bermuda grass suggested that a 1.0% increase in digestion rate resulted in a .67% increase in maximum digestible dry matter intake. Digestion rate can have significant effects on dry matter digestibility (Mertens, 1977). The effect of rate is magnified in grasses with high levels of cell wall content.

Several factors have been suggested which may have an affect on digestion rate. Limited growth and reproduction of microorganisms in the rumen may influence rate because microorganisms produce enzymes necessary for fiber degradation; however, few factors that affect microbial population have been studied for their impact on rate of digestion. Plant composition has an impact on rate of digestion. The morphological types of leaf tissues vary in rate of digestion with the mesophyll and phloem tissue being rapidly digested, while bundle sheaths and epidermis tissue are slowly degraded and the vascular bundles and sclerynchema are almost entirely indigestible (Akin et al., 1974; Akin and Amos, 1975). In addition, individual animals may affect digestion rate through mastication and rumination. It has been reported (Dehoirity and Johnson, 1961) that reducing particle size can increase cell wall digestibility in vitro. The animal also influences rumen pH through buffering capacity. Rumen pH affects the ratio of

species in the rumen microbe population (Russell et al., 1979), and inhibits cellulase activity when pH falls below 6.0 (Stewart, 1977).

Fiber digestion lag time is a period at the initial onset of digestion in which very little digestion occurs. The effect of lag time increases exponentially with increasing levels of fiber in the diet, indicating its importance as a variable in digestion (Mertens, 1977). There have been several explanations to account for the phenomenon of lag time. Brazle and Harbers (1977) completed work that indicated penetration of the epidermal layer may provide an initial barrier to digestion. It may also be possible that physical or chemical inhibitors must be removed or that swelling of fibers is required before enzymes can contact and react with fiber particles. Attachment of bacteria to fiber is necessary for some fiber digestion (Akin, 1979). These suggestions all imply that plant and bacteria interactions are responsible for lag time.

Mertens and Ely (1982) reviewed other explanations for lag time. Period of lag may be due to delay in digestion until bacterial numbers and concentration of fiber-digesting enzymes reach levels that are no longer limiting to digestion. A preference for starch or other readily fermentable carbohydrates may also be a factor involved in lag time. The addition of starch to forage has shown to increase fiber digestion lag time (Mertens and Loften, 1980).

The third component of fiber digestion, extent of digestibility, has been shown to have a great impact on digestibility of forage. In a simulation (Mertens and Ely, 1979), an increase in the indigestible fraction resulted in a decrease in digestibility and an increase in

neutral detergent fiber content of the rumen and intestines. The dry matter intake was also affected and showed a decrease. Values for coastal bermuda grass indicated that a 1.0% decrease in indigestibility resulted in a 1.0% increase in maximum digestible dry matter intake.

Lignin is generally accepted as an anti-quality factor which inhibits utilization of fibrous carbohydrates in forage. Smith et al. (1972) noted correlations of .78 or higher between lignin and the 72 hour cell wall indigestibility used to predict potential digestibility. This relates to work reported by Akin and Amos (1975) in which the indigestible portion of plant material contains high levels of lignin. Waldo and Smith (1979) suggest cellulose is composed of two fractions; potentially digestible and indigestible. The potentially digestible fraction disappears from the rumen by both passage and digestion whereas the indigestible disappears only with passage. Lignin concentration influences extent of digestibility but increasing lignin does not slow rate of digestion.

Passage rate is the fourth component of fiber digestion. When long forage is consumed by an animal, particle size must be reduced before the forage can escape the rumen (Pearce, 1967). Particle reduction in the rumen may be considered as a rate limiting step in the process of forage escape from the rumen (Smith et al., 1983). Prehension, rumination (Welch, 1982), and microbial weakening of fiber (Akin and Amos, 1975) are factors involved in particle reduction. Contribution of microbial action to particle reduction has been questioned. Murphy and Nicoletti (1984) evaluated the particle

size of coarse ground hay after incubation in vitro for 48 hours and indicated minor contribution of microbial action to particle reduction. Conflicting results were reported by Pearce and Moir (1964) in which sheep fitted with devices to inhibit rumination were able to consume a diet of known high rumination potential, and it was concluded that microbial breakdown was sufficient in the rumen for passage of digesta. In a model presented by Mertens (1977) ruminal digestion of grass decreased exponentially as passage rate increased. With a passage rate of 1.0% per hour grass dry matter digestibility was 67.2%, and when rate was increased to 5.0% disappearance per hour the dry matter digestibility value dropped to 58.4%. Studies have shown that grinding a diet to decrease particle size decreases retention time in the rumen (Moore, 1964, Alwash and Thomas, 1971). Retention time has also been effected by feed intake (Grovum and Williams, 1979). As intake increases retention time in the rumen decreases. It is evident that passage rate can be influenced by physical form of the diet, rumination, and to a lesser degree, microbial action, and passage of feed through the rumen can have an effect on digestion.

The relationship of liquid passage and fermentation is receiving more attention. Fermentation responses to change in fluid dilution rate are complex. Bull et al. (1979) suggest that increased liquid turnover may benefit the animal in terms of microbial growth efficiency, improvement in utilization of nonprotein nitrogen and reduction in fermentation losses.

Adams and Kartchner (1984) reported that increased intake in steers consuming a hay diet increased the fluid dilution rate. Fluid dilution rate was 4.3 %/h for steers consuming 1.40% body weight, and 7.2 %/h for those consuming 2.4% body weight. Estell and Galyean (1985) indicate similar results with compiled data collected from seven beef steer trials.

Diet composition is another factor that may influence liquid dilution rate. Owens and Isaacson (1977) related elevation of percent roughage in the diet to increased intake resulting in higher rate of liquid passage. Other studies revealed a decrease in liquid dilution rate when the level of concentrates were elevated in the diet (Cole et al., 1976; Goetsch and Galyean, 1982).

Liquid dilution rate has also been related to ammonia levels and osmolarity in the rumen (Prigge et al., 1984). Estell and Galyean (1985) showed a positive relationship ($P<.01$) existed between ammonia concentration and fluid dilution rate in a trial where beef steers were grazing blue grama rangeland. Effecting fluid dilution rate with hypertonic solutions infused in the rumen was studied by Rogers et al. (1979). Holstein steers fed a high roughage diet were infused with sodium chloride. Fluid dilution rates increased ($P<.05$) from 5.85 %/h with no infusion to 7.11 %/h with infusion of sodium chloride. The increased dilution rate was not accompanied by changes in rumen volume. Increased transruminal water flux accounted for the higher level of liquid required for increased flow rate.

Chapter 3

EXPERIMENTAL PROCEDURE

In Vitro Trials

Nine amino acids, arginine, cysteine, histidine, leucine, lysine, methionine, tryptophan, phenylalanine, and isoleucine, and three branched chain organic acids, isovaleric, isobutyric, and 2-methylbutyric, were evaluated to determine their influence on in vitro fermentation of winter range forage esophageal extrusa. Fermentation rate, digestibility and lag times were calculated for both dry matter (DM) and neutral detergent fiber (NDF) content of fermented samples. Two replications of triplicate samples incubated with either single amino acid or organic acid additions were conducted.

The winter range forage was a composite of esophageal collections from cows grazing winter pastures on the Red Bluff Range Research Ranch located 56 km west of Bozeman, Montana on the northwest slope of the Madison Range. Grasses composed 65 to 75% of the vegetation, with forbs and woody species making up the remaining 25 to 35%. The forage samples were freeze dried, ground through a 1 mm screen and weighed (.25 gram) into 50 ml in vitro fermentation tubes. McDougall's buffer solution (McDougall, 1948) was modified to contain 50 mg nitrogen per 100 ml buffer. Amino acid was added to the buffer to provide 25% of buffer nitrogen with 75% supplied by urea. All amino acids were L-isomers. Branch chain organic acids were added to buffer at 0.1%, and 100% of buffer nitrogen was contributed by urea. Urea supplied all the

buffer nitrogen in the buffer solution for the control. Following pH adjustment to 6.9, 20 ml of buffer were added to the forage sample. Rumen innoculum was obtained from two fistulated crossbred beef cows fed mature grass hay and barley straw ad libitum. Fluid from donor cows was composited and strained through sixteen layers of cheesecloth to remove large digesta particles. The fluid was continuously agitated and maintained at 39° C while 5 ml were innoculated into each in vitro tube. Fermentation tubes were flushed with CO_2, capped, and incubated at 39° C. At 2, 4, 6, 8, 10, 12, 18, 24, 36, and 48 hours postinoculation, fermentation was ceased with the addition of .5 ml 5% mercuric chloride in two blank, triplicate treatment and control tubes. After incubation, tubes were centrifuged at 2000 rpm for 15 min, decanted, dried for 48 hours at 60° C, and weighed to determine DM disappearance. The concentration of NDF was determined for each residue (Van Soest and Robertson, 1980).

Fermentation rates for DM and NDF were determined by nonlinear regression techniques (SAS, 1978). Variable b in the equation, dig. = ae^{bt}, estimated the rate of change of DM or NDF for the entire incubation period. Lag times for DM and NDF were calculated using the first order kinetic equation (Mertens, 1977):

$$\log \frac{A_o}{A} = \frac{K(t-t_L)}{2.303},$$

where A_o = concentration at zero hour, A = concentration at time t, K= rate of disappearance, and t_L = lag time.

Data were subjected to analysis of variance for randomized block design, whereas the replicated in vitro runs were designated as

blocks. When a significant f-value was indicated, means were tested by Least Significant Difference multiple comparison (Snededcor and Cochran, 1980).

In Situ Trial

Methionine additions to in vitro buffers increased ($P<.05$) DM and NDF fermentation rate in comparison to the control and exhibited higher values for DM and NDF digestibility although not significant. Based on the in vitro results methionine was selected for further evaluation. Feed grade methionine is an amino acid commercially available, which could be incorporated into supplements at competitive costs.

Three supplements designated as, DL-methionine-urea, SBM and urea, (table 1) were formulated to provide 1.23 kg total digestible nutrients and .32 kg crude protein equivalence daily, and were evaluated for their influence on forage DM and NDF rumen fermentation. The nitrogen sources for the supplements were DL-methionine, SBM, urea, and beet pulp. DL-methionine provided 4%, urea 63%, and beet pulp 33% of the crude protein in the DL-methionine-urea supplement. Soybean meal contributed 81% of the protein content in the soybean meal supplement and beet pulp supplied 19%. Fifty-three percent of the protein in the urea supplement was derived from urea, 12% from ammonium sulfate, and 31% from beet pulp. Ammonium sulfate was added to the urea supplement to provide 7.9 grams sulfur, an equivalent amount of sulfur as contributed by methionine in the DL-methionine-urea supplement.

Six mature crossbred beef cows fitted with rumen cannulas were utilized in this trial. Each treatment replication (two cows) were confined in one pen and maintained on a forage mix containing 75% mature grass hay and 25% barley straw, chopped by a tub grinder thru a 5 cm screen, and fed ad libitum. The forage was 7.0% crude protein and 62.96% NDF on an as fed basis. Cows consumed approximately 13.33 kg DM of the chopped forage mix daily. The supplements were fed daily at 0800 hour when cows were haltered and tethered to assure each cow consumed all of the daily supplement treatment. The amount of supplement fed per head per day was as follows: 1.95 kg DL-methionine-urea supplements, 1.97 kg urea supplement, and 1.85 kg SBM supplement. Each supplement supplied .32 kg protein and 1.23 kg total digestible nutrients. The experimental periods allowed for 10 days of adaptation to supplements followed by a five day sample collection period.

Bags were made of nylon monofilament material with a pore size of 44 microns. Each nylon bag was double stitched around the bag perimeter and the outer dimensions were 15.2x17.8 cm. The sample fermented in the nylon bags was identical to the chopped forage fed to the cows. The forage was ground by a Wiley mill through a 2 mm screen and three gram samples were placed in each bag. This allowed for a ratio of 7 mg of sample per cm^2 of nylon material (Uden and Van Soest, 1984). Bags were attached to snap swivels on a hoop of tygon tubing filled with a chrome ball chain for suspension Rumen digesta collected from cannulated cows fed the diet previously described was prepared as a mordant according to Uden et al. (1980) and contained

3.27% chromium on the fiber. The mordanted fiber served as a particulate passage marker in the rumen. Cobalt EDTA was prepared as a liquid passage marker in the rumen (Uden et al., 1980).

At zero hour on day one of the collection periods, each cannulated cow was dosed with 100 g of chromium mordanted fiber and 15 g cobalt EDTA. The bags were suspended in the rumen and 15 minutes later rumen contents were sampled. All rumen samples were grab samples (500 ml) from approximately the same location in the cranial sac of the rumen. Collection times were 4, 8, 12, 18, 24, 36, 72, and 96 hours. For each time period, two sample filled bags and rumen extrusa were collected from every cow, and one blank bag was collected from one cow on each treatment.

Rumen samples were immediately treated with 1 ml 5% mercuric chloride to cease any further microbial activity, centrifuged at 2000 rpm for 15 minutes, the fluid was decanted, and both fluid and fiber samples were frozen at -4° C for later analysis. The nylon bags were washed with cold water until the rinse water was clear when squeezed out of the bag. The bags were dried in a forced air oven at 60° C for 48 hours.

Dry matter and NDF (Van Soest and Robertson, 1980) content was determined for each bag. Rumen digesta samples were prepared (Williams, David and Iismaa, 1962) and the chromium concentration was determined with atomic absorption spectrophotometry. Rumen fluid samples were analyzed for cobalt and rumen ammonia concentration. A portion of each fluid sample was centrifuged at 10,000 rpm for 10 minutes and the supernatant was evaluated for cobalt concentration

with an atomic absorption spectrophotometer (Gaylean and McCollum, 1985). Rumen ammonia levels (mg/100 ml rumen fluid) were determined by a macro Kjeldahl procedure (AOAC, 1980).

Dry matter and NDF disappearance values were subjected to nonlinear regression (SAS, 1985) to estimate rate of fermentation for each parameter. The equation used for the nonlinear regression analysis was; $Y = ae^{[-k\ (t-tlag)]} + u$, where as Y = digestibility, a = potential digestibility, -k = rate of fermentation, t = time, tlag = lag period, and u = undigestible fraction. Dry matter data did not indicate a period of fermentation lag, therefore the tlag variable was not included in the DM equation for fermentation parameters estimates. Cobalt and chromium concentrations were transformed into natural logarithms and linear regression techniques (Snedecor and Cochran, 1982) were applied to determine the rate of passage from the rumen of the liquid and particulate fractions.

Dry matter and NDF rumen digestibilities of the forage sample were calculated with the following equation:

$$\text{Rumen Digestibility} = 100 - [a\ (k_1/(k_1+k_2)) + b\ ],$$

a = potential digestibility, k_1 = particulate rate of escape from the rumen, k_2 = fermentation rate, and b = undegradable portion. The potential digestibility value used for calculation was the percent digestible at 72 hours.

The liquid dilution rate constant was utilized to predict three liquid parameters of the rumen, fluid volume, fluid outflow, and fluid turnover time as influenced by the supplements. Fluid volume was estimated using the equation, volume = (ml/a)(b), whereas a =

concentration of cobalt at zero hour (intercept of the linear regression line) and b = amount of cobalt EDTA given at time zero hour. The equation, outflow = liquid volume/ escape rate, provided a measure of fluid outflow from the rumen. The third parameter, fluid turnover time, equals the inverse of the rate constant.

The experimental design of this trial was a 3x3 replicated Latin-square; whereas, over the course of the three periods all cows received each treatment. All estimates were subjected to analysis of variance for replicated Latin-squares (Snedecor and Cochran, 1980). When a significant f-value was indicated then mean differences were determined by the Newman-Keuls means test.

Heifer Growth Trial

Four isonitrogenous isocaloric supplements (table 1) incorporated into roughage diets fed to weanling heifer calves were evaluated by performance measurements. Three supplements, DL-methionine-urea, SBM and urea, were formulated similar to those described in the in situ trial. In the fourth supplement, soybean meal-urea, urea contributed 47%, soybean meal 28%, and beet pulp 25% to the protein content. The supplements and a chopped roughage mixture consisting of 75% mature grass hay and 25% barley straw, were used to formulate rations to meet Nutritional Research Council requirements (NRC, 1976) for heifers gaining .3 kg per day.

The feeding trial was conducted at the Montana Agriculture Experiment Station Feedlot, Bozeman. Fifty-six weanling heifer calves with an average initial weight of 231.5 kg were used in the feeding

trial. Breeds of the heifers included Angus, Hereford, Angus-Hereford crossbred, and Tarantaise crossbred. Initial weights were an average of two consecutive day weights. The heifers were randomly assigned to groups within breed and body weight stratification. Four pens were allotted to each treatment group; two of the pens held four heifers while the other two pens contained three. The pens had fence line bunks protected by partial roof cover, and were bedded with straw. Automatic waterers provided water and trace mineral salt blocks were available in the bunks for each pen.

The heifers were fed once daily in the morning. Diets were mixed individually by treatment in a feed wagon equipped with a scale to weigh the forage. For those diets containing urea, the urea was dissolved in 11.748 liters of water and sprayed over the ration as it was mixing in the feed wagon. This procedure allowed for a more even distribution of the urea and reduction in palatability problems. Water without urea was sprayed on the SBM diet in the same fashion. Feed remaining in the bunks was removed and weighed every other day to estimate DM intake. When heifers were consuming all of their diets the forage level in the ration was increased, however the amount of supplement remained constant throughout the entire feeding period. The supplements were fed to provide the following levels per head per day: 1.95 kg for the DL-methionine-urea treatment, 1.97 kg for the urea treatment, 1.68 kg for the SBM treatment, and 1.85 kg for the SBM-urea treatment.

The duration of the feeding trial was ninety days. During the trial the heifers were weighed every fourteen days before the morning

feeding to monitor animal performance. Water was withheld for twelve hours prior to weighing time. A final weight was an average of weights collected on the last two days of the trial. Intake, daily gain, and feed efficiency were calculated as indications of performance. Individual animals were used as the experimental unit for average daily gain while pen was the experimental unit for intake and feed efficiency. Data was analyzed by analysis of variance for complex randomized block designs utilizing y = treatment, pen(treatment) as the model (SAS, 1985).

Chapter 4

RESULTS

In Vitro Trial

Extent of in vitro fermentation at 48 hours (table 2) for all amino acid, arginine, cysteine, histidine, leucine, lysine, methionine, tryptophan, phenylalanine, and isoleucine, and branch chain organic acid, isobutyric, isovaleric, and 2-methylbutyric, treatments in comparison to forage alone was similar ($P>.05$). The control, containing no amendments, had 41.2% disappearance of DM at 48 hours. Other dry matter disappearance values ranged 36.9% (isobutyric acid) to 47.4% (methionine). The addition of arginine (47.1%) or methionine (47.4%) improved ($P<.05$) disappearance when compared to isobutyric acid, lysine or phenylalanine (36.9, 37.1, and 39.9%, respectively). It is interesting to note that the control had the third lowest disappearance extent (41.5%) which was nearly six percentage units lower than either arginine or methionine addition.

Dry matter fermentation rate (table 2) was increased ($P<.05$) by over 30% with addition of arginine, methionine, histidine, leucine, or cysteine (3.6, 3.6, 3.5, 3.5, and 3.4 %/h, respectively) in comparison to forage alone (2.7 %/h) or lysine (2.6 %/h) addition. Tryptophan, isoleucine, phenylalanine, isobutyric, leucine and 2-methylbutyric (3.1, 3.3, 2.8, 2.9, 3.0, and 3.1 %/h, respectively) were similar ($P>.05$) to forage alone. Linear regression analysis indicated a

positive ($P<.01$) correlation ($R^2 = .78$) between extent and rate of dry matter forage.

Neutral detergent fiber 48 hours disappearance (table 3) was similar ($P>.05$) for all amendments in comparison to the control with no amendments. The addition of histidine, arginine, leucine, methionine, isoleucine, isovaleric and 2-methylbutyric acid (57.8, 56.9, 54.5, 56.2, and 56.6%, respectively) improved NDF disappearance in comparison to phenylalanine (47.9%). Tryptophan, phenylalanine, lysine, isobutyric acid, and forage alone consistantly ranked numerically among those treatments with the least DM and NDF fermentation extent. Arginine, histidine, methionine, and isoleucine appeared in the four treatments which ranked highest for both DM and NDF digestion extent. NDF analysis of the winter range forage indicated the sample contained 67.9% NDF which may account for similar trends noted between DM and NDF data.

The rate of NDF fermentation (table 3) for the control was numerically the slowest (2.5 %/h) of all treatments tested, which was slower ($P<.05$) than cysteine, methionine, and leucine (3.8, 3.8, and 3.4 %/h). These amendments improved NDF fermentation rate by 52, 52, and 36%. Linear regression analysis showed no significant correlation between NDF fermentation extent and rate.

Lag times calculated for DM and NDF fermentation appear in table 4. Statistical analysis revealed no significant differences between treatments for DM or NDF lag times. The estimated lag time for in vitro digestion of the DM ranged from 28.0 hours (control) to 32.1

hours (isobutyric acid). Neutral detergent fiber lag time values were between 20.2 hours (lysine) and 25.2 hours (methionine).

In Situ Trial

Dry matter fermentation rates (table 5) were affected in a similar fashion. Fermentation rate was 9.54 %/h when cows received DL-methionine-urea supplement which is an improvement ($P<.05$) compared to 7.28 %/h for SBM and 7.74 %/h for urea supplementation. The values indicate that the DL-methionine-urea supplement increased fermentation rate 29 and 31% in comparison to rates determined when cows received the SBM or urea supplements.

Supplements also had an influence ($P<.05$) on particulate passage rate from the rumen (table 5). Digesta particulate passage rate when animals received the SBM supplement (4.92 %/h) was approximately sixteen percent more rapid than passage rate for urea (4.24%) and DL-methionine-urea (4.20%) supplementation.

The estimated DM rumen digestibility (table 5) of the forage was influenced by the supplements. The calculated rumen digestibility was 48.49% for the DL-methionine-urea treatment and 47.55% for the urea treatment which was higher ($P<.10$) than than the digestibility value, 42.40%, for the SBM treatment. In comparison to SBM, DL-methionine-urea and urea supplements increased rumen digestibility of DM 14.4% and 12.1% respectively.

The results for NDF fermentation rate and rumen digestibility (table 6) followed similar trends as the DM fermentation measurements however no differences ($P<.05$) could be detected. Rumen NDF

digestibility was increased by DL-methionine-urea treatment (39.21%) in comparison to urea (35.28%) and SBM (36.53%) treatments. Neutral detergent fiber fermentation rate was improved 21 to 28% when cows were supplemented with DL-methionine-urea (5.13 %/h). Fermentation rate was 4.01 %/h when cows received SBM supplement, and 4.24 %/h when urea was supplemented.

Two of four rumen liquid parameters were influenced by supplements (table 7). Rumen fluid dilution rate was increased ($P<.01$) with DL-methionine-urea (10.62 %/h) and urea (10.53 %/h) supplements when compared to SBM supplement (9.77 %/h). Supplements had a similar effect on fluid turnover time. During the trial when cows were supplemented with SBM the fluid turnover time, 10.25 h, increased ($P<.01$) in comparison to DL-methionine-urea (9.50 h) and urea (9.56 h) supplementation.

Fluid volume was similar between all treatments; 40.91 liters for DL-methionine-urea, 41.75 liters for SBM and 41.86 liters for urea. Outflow of fluid from the rumen was also not affected by the different supplements. Fluid outflow values reported are 3.89, 4.28, and 4.0 liters per hour for Dl-methionine-urea, SBM and urea supplements, respectively.

Rumen ammonia levels (table 8) are reported for each collection hour and an average for the entire trial. Ammonia levels are not significantly different for treatments. Over the entire trial, the rumen ammonia levels were 9.53, 8.26 and 9.22 mg/100 ml rumen fluid respectively for the following treatments, DL-methionine-urea, SBM and urea. Ammonia levels at the 4 hour collection was higher ($P<.05$)

with DL-methionine-urea and urea supplements (26.38 and 25.72 mg/100 ml) compared to SBM (11.01 mg/100 ml).

Heifer Growth Trial

Average daily gain (table 9) for heifer calves consuming a low quality forage was similar for all supplements, DL-methionine-urea, SBM, urea, and SBM-urea. The average daily gains for the supplemental regimes were .60 kg (DL-methionine urea), .64 kg (SBM) .62 kg (urea) and .60 kg (SBM-urea). The feed intake (table 9) is reported as an average intake per head per day and indicates no difference between treatments. Feed intake over the entire feeding trial was 6.83, 6.74, 6.74, and 7.29 kg/(h d^{-1}) for DL-methionine urea, SBM, urea, and SBM-urea supplementation. The third measurement calculated, feed efficiency (table 9), was also not influenced by the treatments. Feed efficiency (kg DM ingested /kg gain) was 11.44, 10.71, 11.75, and 11.26 for DL-methionine , SBM, urea, and SBM-urea supplemented heifers, respectively.

Chapter 5

DISCUSSION

In Vitro Trial

The association of various fermentation factors such as rate, digestibility extent of potentially digestible fraction, lag time and rumen passage rate all effect digestibility in the animal. An understanding of the dynamics of these factors in relation to winter range forage utilization may prove beneficial. The in vitro technique provided results for use as a screening technique; however, there are limitations in evaluating digestion extent and the impact of digesta passage rate. In situ methods are more adapted for evaluating digestion extent and rumen passage rate simultaneously.

Results from the in vitro trial revealed trends that provide a strong basis for future research. Mertens (1977) indicated rate can have significant impact on DM digestibility especially in grasses with high cell wall contents. This study presented significant DM rate differences and a positive correlation between rate and extent of digestion.

Lag times determined in this study were longer than 20 hours. The duration of lag time was much longer than anticipated when compared to previous work with other forages (Mertens and Loften, 1980; Simpson, 1984) where lag time was reported to be four to five hours. Graphs (figures 1-3) of the indigestible DM portion plotted against time reveal three phases of fermentation: a rapidly

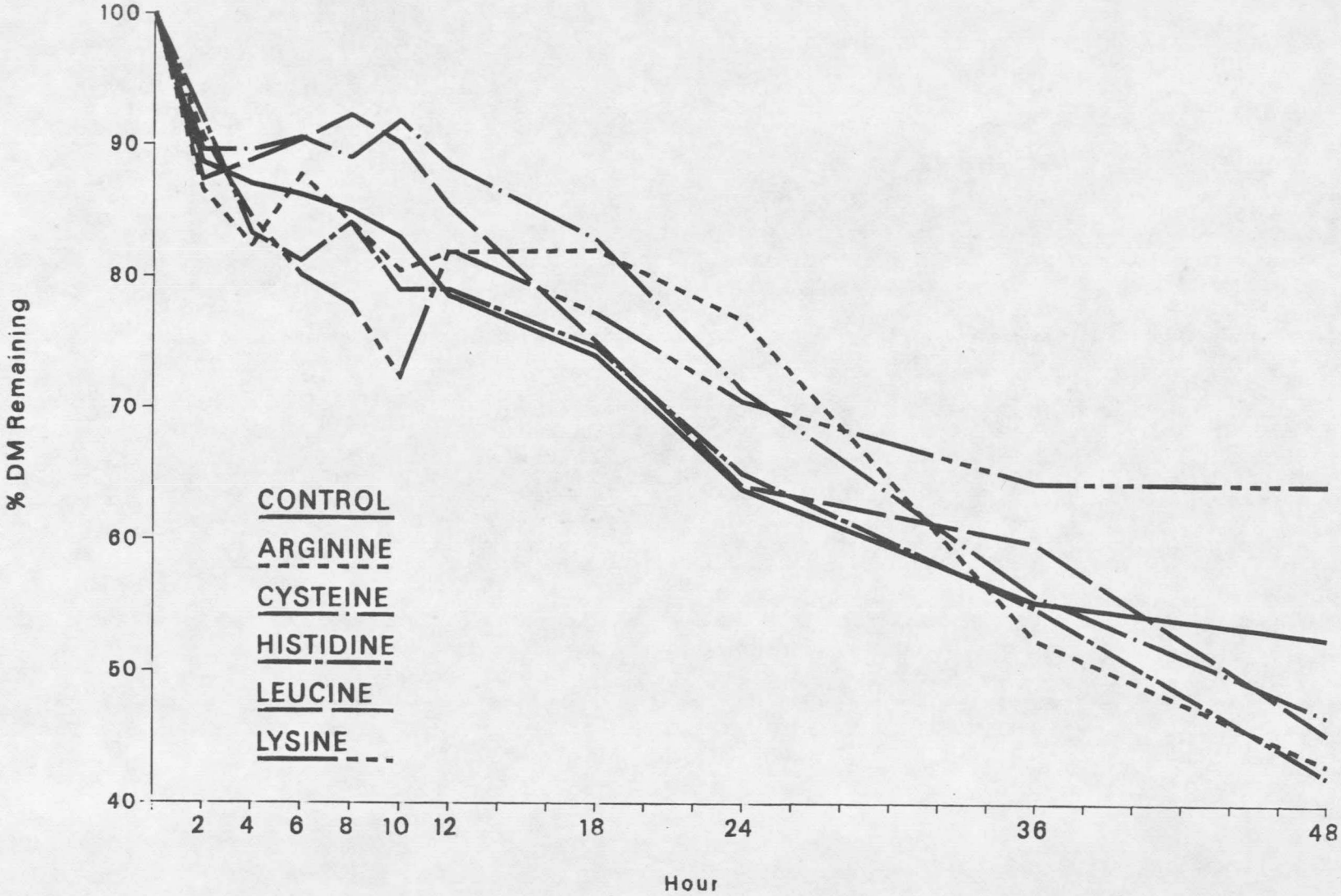

Figure 1. In vitro dry matter digestibility of native winter range forage

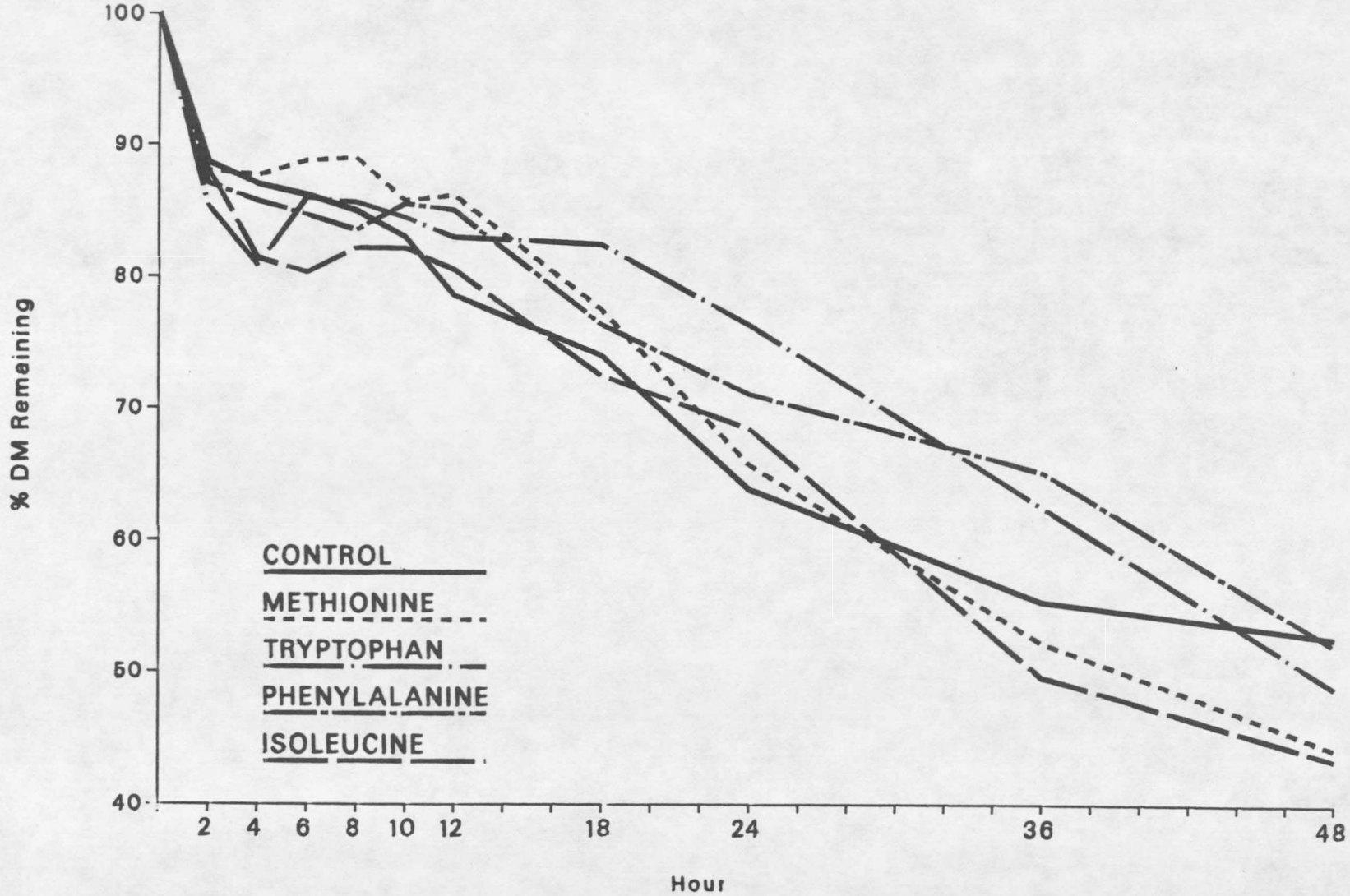

Figure 2. In vitro dry matter digestibility of native winter range forage

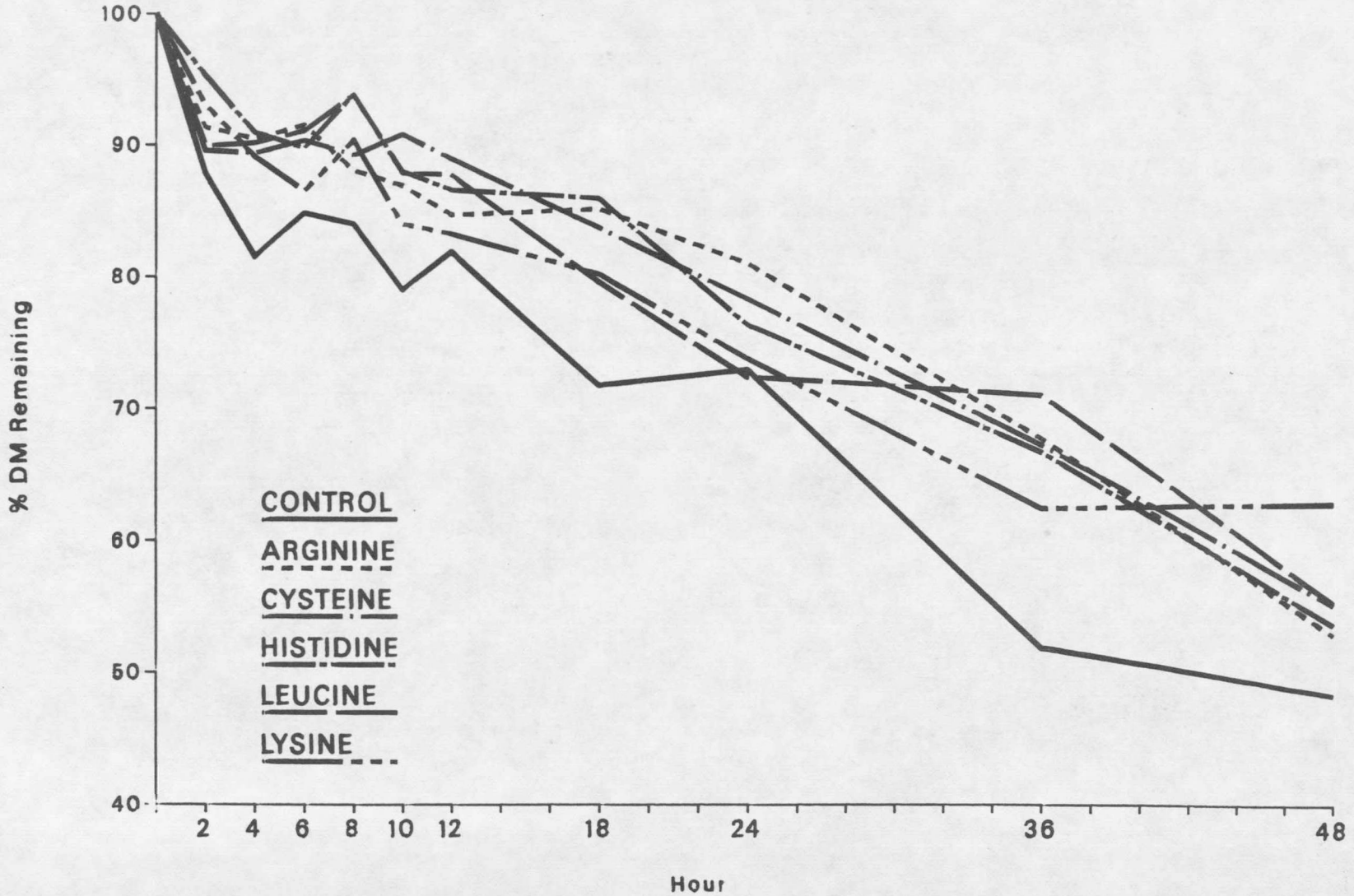

Figure 3. In vitro dry matter digestibility of native winter range forage

degradable phase in which the cell solubles are released, a slow phase indicating lag time for the non-soluble fraction, and a fermentation phase in which the rate increases with breakdown of the less degradable fraction. Graphs (figures 4-6) of NDF fermentation reveal similar phases; however, the second phase is more pronounced indicating a more definite lag period for NDF in comparison to DM. Mertens (1977) reported cellulose fermentation rates of 8-16 %/h for readily digested cell wall parts and 2-3 %/h for the slowly digested fractions indicating rate is not constant during the fermentation process. The value of 2-4 %/h for slowly digested fractions is in agreement with the in vitro NDF rates which had a 2.5-3.4 %/h range.

The lag times reported where calculated assuming 100% potential digestibility of the forage, which would increase the lag time. A more realistic lag time could be obtained if potential digestibility of the forage was known. Thus amount digested for a given incubation period could be compared directly to the potentially digestible fraction. This supports the need for determining potential DM and NDF digestibility for the winter range forage esophageal extrusa samples in order to improve lag time estimates.

The effect of lag time on fermentation increases at an exponential rate according to the model outlined by Mertens (1977). This model indicates that lag time may be an extremely important variable in fermentation. The elements responsible for lag time are not well documented. There are several possible causes for lag time: physical characteristics of the forage, bacterial characteristics,

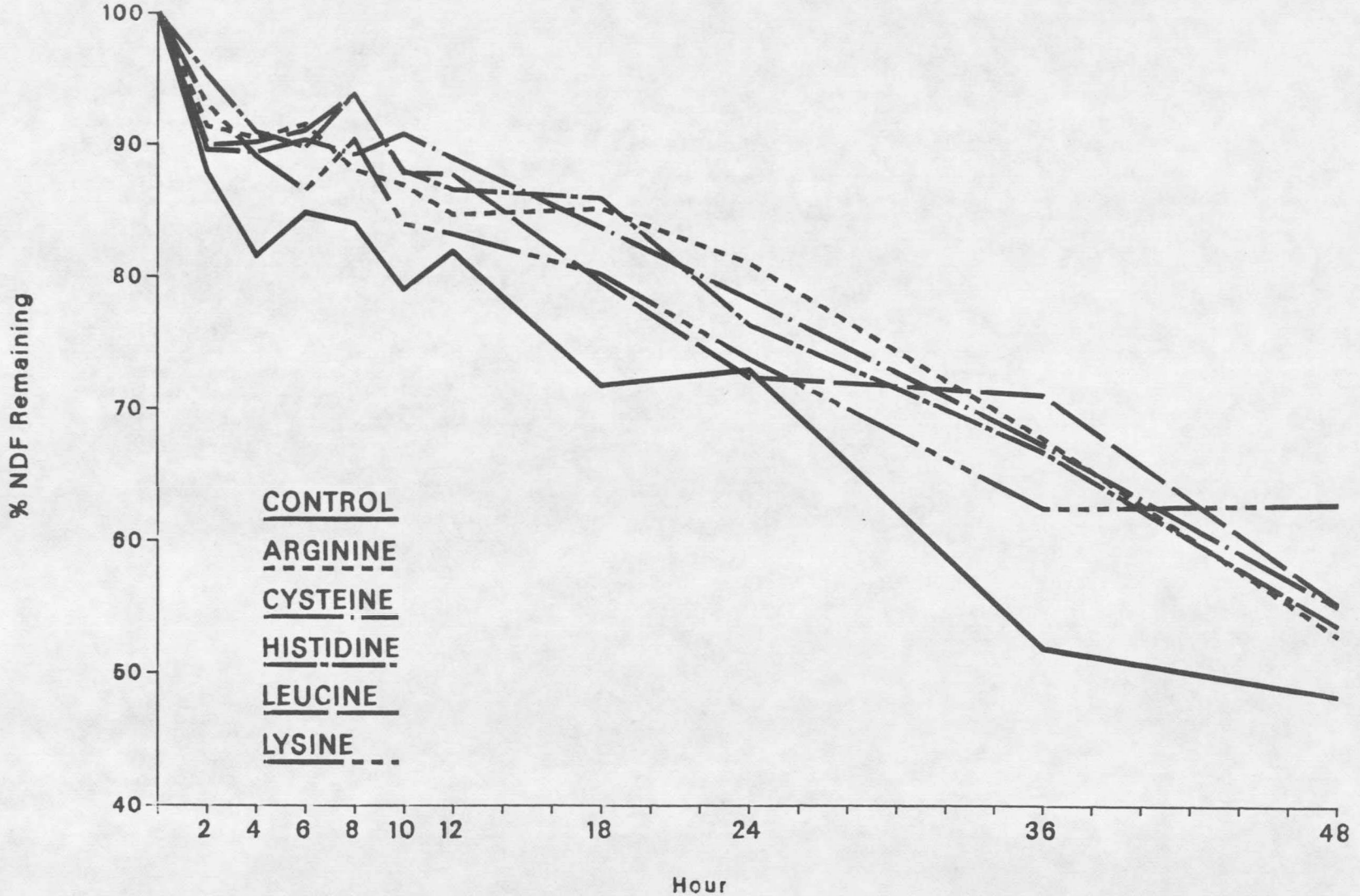

Figure 4. In vitro neutral detergent fiber digestibility of native winter range forage

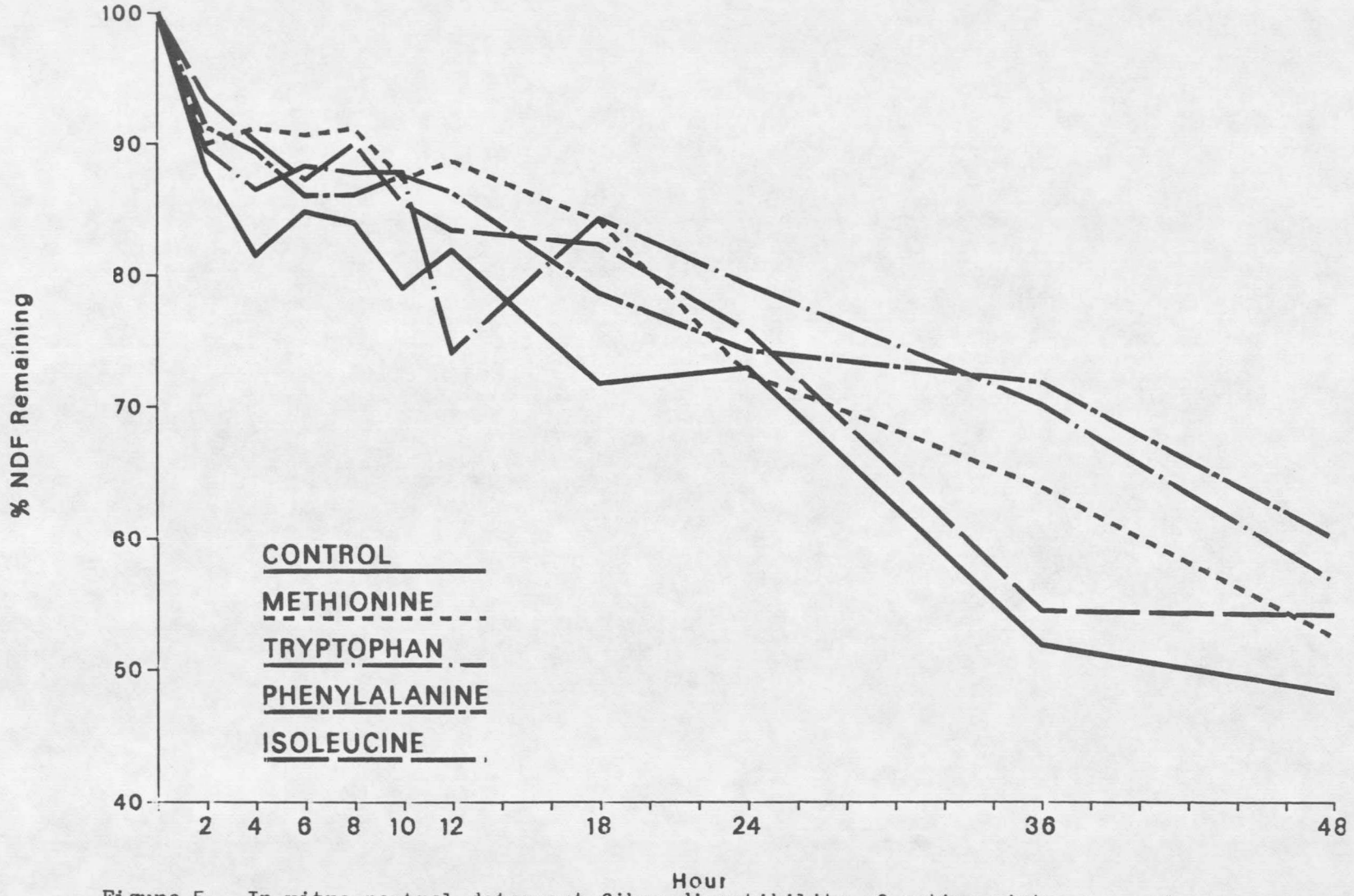

Figure 5. In vitro neutral detergent fiber digestibility of native winter range forage

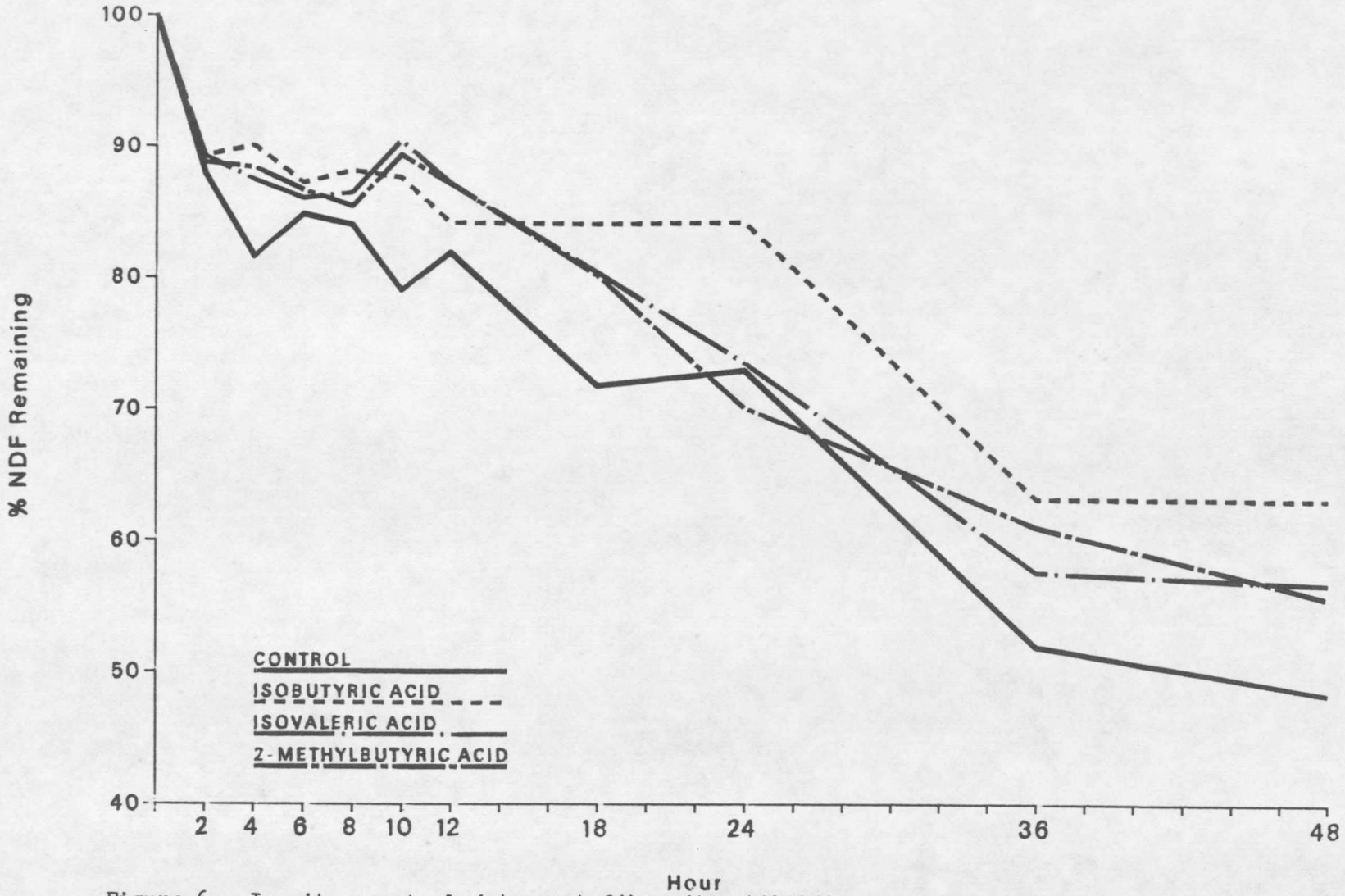

Figure 6. In vitro neutral detergent fiber digestibility of native winter range forage

polarity of bacteria and forage particle or chemical reactions that may be involved.

In Situ Trial

Results of the in situ trial agree with those of the in vitro trial. Methionine had a positive influence on fermentation rate both in vitro and in situ. Salter et al. (1979) determined methionine to be a limiting amino acid for bacteria on diets low in protein. Providing methionine to rumen bacteria may remove this limitation, allowing for better bacterial growth and rate of fermentation. When comparing results from supplementation, DL-methionine-urea increased DM fermentation, particulate passage rate increased with SBM supplementation, and both DL-methionine-urea and urea improved rumen digestibility and had similar particulate passage rate. This implies that passage rate has a more influential effect on rumen digestibility than fermentation rate and may help to explain why no digestibility differences were found in the in vitro trial when fermentation rate was significantly influenced. Hoover et al. (1982) reported DM and acid detergent fiber digestibilities increased with increasing retention of solids in a continuous culture device fermenting a semipurified diet with zein as the major protein source.

The in situ NDF results followed similar trends as DM but no significant differences were detected as in the in vitro trial where methionine significantly increased NDF fermentation rate. This may be due to laboratory technique involved in determining the NDF content of the sample in the nylon bags. Neutral detergent fiber was determined

by refluxing the bag and sample in NDF solution. Individual bags were rinsed manually with hot water. A more uniform method for rinsing the soap out of the sample and nylon material could reduce experimental error and provide more accurate NDF values.

Dry matter fermentation in situ exhibited no lag period and lag times were not included in the rate equation. The lag period for NDF was approximately one hour for each of the treatments. A lag period is not evident in DM fermentation because while lag is occurring in the NDF fraction, the readily soluble portion is being released. These results are quite different from lag times calculated for in vitro fermentation, and as mentioned previously the in vitro lag times were based on 100% potential digestibility of the forage sample. The in situ results are more in agreement with other reported lag times and strongly supports the need for determining the potential digestibility of forage samples in order to estimate realistic lag times. Varga and Hoover (1983) calculated a .90 hour lag period for orchard grass in situ.

From the results it appears that particulate and liquid passage rates were influenced independmntly. The passage of the particulate fraction was 17% slower ($P<.05$) for both DL-methionine-urea and urea supplementatin compared to SBM. Particulate pasSage rate has been shown to be influmnced by intake, as intake increases so does the pavticulate passage rate (Owens and Isaacwon, 1977). It is feasible that intake may be driven by metabolic eneroy needs of an animal.

Maintenance energy expenditures were found to be highly correlated to body protein mass maintenance (Ferrel et al., 1979).

Energy expenditure for daily protein comprised 89% of the basal energy expenditure. In a later study, Ferrel and Jenkins (1985) concluded after several different approaches that metabolism of visceral organs constitute a major proportion of total animal energy expenditures. Results observed by the same scientists indicated weight of the organs can vary in response to nutritional treatments. Animals on a higher plane of nutrition had heavier weights for the stomachs, small intestines, large intestines, livers and kidneys. Animals in a positive nitrogen balance may require more energy to maintain current protein mass compared to an animal in a negative nitrogen balance. The higher maintenance energy requirement possibly could influence intake in that the animal consumes more to meet its needs.

Bypass protein can place an animal in a state of positive nitrogen balance. Cows on winter range receiving a blood meal protein supplement had higher ($P<.05$) intake, 1.46 % of body weight, than cows not receiving the supplement, 1.17 % of body weight, (unpublished data, Dunn, 1984; Miner, 1984). Cows on the blood meal supplement could be in a higher positive nitrogen balance than those receiving no supplement, and this may support the ideathat maintenance energy requirement to meet protein body mass maintenance may influence intake.

When cows on the in situ trial consumed the urea containing supplements, they could be under nitrogen balance stress compared to the period when cows consumed the SBM. Supplementation with SBM did increase passage rate compared to supplementation with DL-methionine-urea and urea. In consideration of the previous discussion, the

influence of supplement treatments on increased passage rate could be a result of intake driven by energy maintenance requirement.

DL-methionine-urea and urea supplementation increased fluid dilution rate 8.7% ($P<.01$) when compared to SBM. The increase in rate may be explained by the concentration of ammonia in the rumen contributed by urea in the DL-methionine-urea and urea supplements. Ammonia levels in the rumen have been noted as an influential factor for fluid dilution rate (Prigge et al., 1984; Estell and Galyean, 1985). Rogers et al. (1979) was able to affect fluid dilution rate by infusing a hypertonic solution into the rumen.

It may be possible that the ammonium ions affect the osmolarity in the rumen which changes the transruminal water flux providing more metabolic water. The liquid passage increases to dilute ammonia levels so osmolarity is returned to normal. Faster passage rates noted for the urea containing diets could also explain why ammonia levels were the same for all three supplements.

Rumen fluid volume was approximately 41.0 liters and was not influenced by treatments. These values are similar to rumen volume (55 l) reported by Rogers et al. (1979), but lower than volume values (130 l) reported by Adams and Kartchner (1984). All compared values are for animals consuming a high roughage diet. It is not uncommon to observe similar rumen volume when turnover is altered (Harrrison et al., 1975) The rumen turnover time differences correspond to liquid dilution rate, as dilution rate increases the turnover time decreases.

The rumen ammonia levels were different ($P<.05$) only at the four hour collection period. Supplementation with DL-methionine-urea and

urea significantly increased ammonia level in comparison to soybean meal. The four hour collection was the only collection closely following supplementation as all other morning collections occured prior to supplementation. The significant difference found indicates the DL-methionine-urea and urea supplements influenced ammonia levels in the rumen at four hours post-supplementation. This increase would reflect a more rapid ammonia release from urea contained in the supplements in comparison SBM. Eight hours after supplementation no differences were observed.

Rumen ammonia levels for optimum microbial growth have been reported to be between 5 and 9.0 mg / 100 ml rumen fluid (Hume et al., 1970; Satter and Slyter, 1974; Kang-Meznarich and Broderick, 1981; Pritchard and Males, 1982). Mehrez et al. (1977) indicated a much higher level of 23.2 mg / 100 ml rumen fluid necessary to maximize microbial growth. The ammonia levels for the entire in situ trial were adequate for microbial yield in comparison to the first values listed above.

Ammonia levels do vary among collection periods and no consistant pattern is evident. The cows were fed the forage ad libitum and ingestion of the feed could occur at any time. Wohlt et al. (1976) demonstrated time after feeding influenced ammonia levels. If cows were consuming the forage at various uncontrolled times in relation to collection periods, time after feeding could account for variation in ammonia levels for the trial.

Growth Trial

Average daily gain, feed intake and feed efficiency were not influenced by the supplements, DL-methionine-urea, SBM, urea or SBM-urea fed to heifer calves consuming a low quality forage. The forage intake was not limited for heifers on the trial and they consumed approximately 7.0 kg / (h d^{-1}), which was 2.6 kg / (h d^{-1}) more than had been the anticipated. The high intake values could possibly be attributed to the forage being chopped. Alwash and Thomas (1971) reported that grinding a diet decreases retention time in the rumen and an increase in intake occurs.

Even though the crude protein value was only 7.0%, the heifers consumed enough roughage to almost meet their protein requirements. To gain .3 k/day, the NRC (1976) requirement for protein is .42 kg /day and the heifers were receiving approximately .67 kg/day. The excessive protein provided to the heifers made it difficult to detect any differences in performance measurements due to supplemental protein source. Feeding the supplements while limiting intake may have produced a response due to supplement treatment.

In summary, methionine had an influential effect on fermentation rate of fiber in vitro and in situ. The in situ data indicated the importance of passage rate for determining digestibility of forage. Passage rate had a larger impact on digestibility than fermentation rate. The particulate passage rate and liquid dilution rate appeared to be independently affected. Providing amino acids to rumen bacteria have shown to affect fermentation rate of DM and NDF; however, passage rate appeared to have a greater impact on digestibility.

Chapter 6

CONCLUSIONS AND RECOMMENDATIONS

In vitro and in situ results of this study indicate that methionine influenced fermentation rate of low quality roughage. In both systems, the addition of methionine enhanced fermentation rate by at least 30%. Methionine has been identified as a limiting amino acid for rumen bacteria fermenting low protein diets. Providing readily available methionine to rumen bacteria may satisfy the methionine requirement; therefore, increasing bacterial growth rate. The fermentation rate response to methionine may be a result of increased microbial growth rate.

Particulate and liquid passage rates measured during the in situ trial were inversely affected by treatments. DL-methionine-urea and urea supplementation increased fluid passage rate and decreased particulate passage rate compared to SBM. The increase in fluid passage rate for urea containing supplements may be attributed to a change in rumen osmolarity due to higher ammonia concentration following morning supplementation. The decrease in particulate passage rate is not easily explained. Protein supplementation may have changed body protein mass. As protein mass increased, maintenance energy requirements also increased. This difference may have caused a metabolic stimulation of intake which in turn increased particle passage rate; however, further research is required to determine if this is occurring.

Calculated rumen digestibility was lower for the DL-methionine-urea and urea supplementation in comparison to SBM. Even though DL-methionine-urea supplementation increased fermentation rate, the slower particulate passage rate appeared to have a greater impact on calculated digestibility. Supplements containing urea affected passage rates and digestibility in a similar manner indicating that urea may have an independent impact on rumen activity. Comparing supplemental methionine to a natural protein source such as soybean meal may be difficult in the presence of urea.

The heifer growth trial revealed no differences in daily gain, intake or feed efficiency due to supplements. The method of feeding chopped roughage ad libitum with a constant level of supplement allowed heifers to consume 112% of the protein necessary for a .3 kg daily gain. Differences between supplements may have been detected had the heifers been limited in protein intake.

The following is a list of recommendations that may be considered if research is continued in this area.

1) Bacterial growth rate should be measured to determine if the increase in fermentation rate with the addition of methionine is due to an increase in bacterial growth rate.

2) The in vitro technique could be used to determine an optimum level of methionine for enhancing fermentation of low quality roughages.

3) The method of rinsing nylon bags especially during the determination of neutral detergent fiber could be improved to help decrease experimental error and to obtain more

accurate values. Rinsing the nylon bags needs to be more consistant.

4) Intake values for animals used during the in situ trial would have been useful to determine if intake was influencing the particulate passage rate.

5) Ammonia concentration in the rumen should be measured more regularly rather than at bag collection time. This would allow a better understanding of differences in ammonia concentration due to supplemental treatments.

6) Methionine supplementation without urea may have different responses that would enhance the utilization of low quality roughages.

7) Feeding a long roughage rather than one which has been chopped would possibly show more of a benefit from increased fermentation rate in the rumen.

8) If a second heifer growth trial were to be conducted the level of protein should be limited to determine if the supplements influences weight gain and feed efficiency.

Results from this study do indicate that the addition of methionine can increase fermentation rate. Further evaluation is needed to better understand the impact of methionine supplementation on rumen dynamics and animal performance on low quality roughages. It may become economically feasible to incorporate methionine into protein supplements for improved animal production.

REFERENCES CITED

REFERENCES CITED

Adams, D. C. and R. J. Kartchner. 1984. Effect of level of forage intake on rumen ammonia, pH, liquid volume and liquid dilution rate in beef cattle. J. Anim. Sci. 58:708.

Akin, D. E. 1979. Microscopic evaluation of forage digestion by rumen microorganisms - a review. J. Anim. Sci. 48:701.

Akin, D. E. and H. E. Amos. 1975. Rumen bacterial degradation of forage cell walls investigated by electron microscopy. Appl. Microbiol. 29:692.

Akin, D. E., D. Burdick and G. E. Michaels. 1974. Rumen bacterial interrelations with plant tissue during degradation revealed by transmission electron microscopy. Appl. Microbiol. 27;1149.

Allison, M. J. 1969. Biosynthesis of amino acids by ruminal microorganisms. J. Anim. Sci. 29:797.

Allison, M. J. 1978. Production of branched-chain volatile fatty acids by certain anaerobic bacteria. Appl. Environ. Microbiol. 35:872.

Allison, M. J., M. P. Bryant and R. N. Doetsch. 1962a. Studies on the metabolic function of branched-chain volatile fatty acids, growth factors for Ruminococci. J. Bact. 83:523.

Allison, M. J., M. P. Bryant, I. Katz and M. Keeney. 1962b. Metabolic function of branched-chain volatile fatty acids, growth factors for Ruminococci. J. Bact. 83:1084.

Al-Rabbat, M. F. and R. L. Baldwin, and W. X. Weir. 1971. In vitro nitrogen-tracer technique for some kinetic measures of ruminal ammonia. J. Dairy Sci. 54:1150.

Alwash, A. H. and P. C. Thomas. 1971. The effect of the physical form of the diet and level of feeding on the digestion of dried grass by sheep. J. Sci. Food Agr. 22:611.

AOAC. 1980. Official Methods of Analysis. (13th Ed.) Association of Official Agricultural Chemists, Washington, D.C.

Bentley, O. G., R. R. Johnson, R. V. Hershberger, J. H. Cine and A. L. Moxon. 1955. Cellulolytic-factor activity of certain short-chain fatty acids for rumen microorganisms in vitro. J. Nutr. 57:389.

Brazle, F. K. and L. H. Harbers. 1977. Digestion of alfalfa hay observed by scanning electron microscopy. J. Anim. Sci. 46:506.

Bryant, M. P. 1973. Nutritional requirements of the predominant rumen cellulolytic bacteria. Fed. Proc. 32:1809.

Bryant, M. P. and I. M. Robinson. 1962. Some nutritional characteristics of predominant culturable ruminal bacteria. J. Bacteriol. 84:605.

Bull, L. S., W. V. Rumpler, R. F. Sweeney and R. A. Zinn. 1979. Influence of ruminal turnover on site and extent of digestion. Fed Proc. 38:2713.

Cole, N. A., R. R. Johnson, F. N. Owens, and J. R. Males. 1976. Influence of roughage level and corn processing method on microbial protein synthesis by beef steer. J. Anim. Sci. 51:975.

Crampton, E. W. 1957. Interrelationships between digestible nutrient and energy content, voluntary dry matter intake and the overall feeding value of forages. J. Anim. Sci. 16:546.

Dehority, B. A. 1968. Mechanism of isolated hemicellulose and xylan degradation by cellulolytic rumen bacteria. Appl. Microbiol. 16:781.

Dehority, B. A. and R. R. Johnson. 1961. Effect of particle size upon the in vitro cellulose digestibility of forages by rumen bacteria. J. Dairy Sci. 19:268.

Dehority, B. A., H. W. Scott and P. Lowaluk. 1967. Volatile fatty acid requirements of cellulolytic rumen bacteria. J. Bact. 94:537.

Eadie, J. M. 1962. The development of rumen microbial populations in lambs under various conditions of management. J. Gen. Microbiol. 29:563.

Estell, R. E. and M. L. Galyean. 1985. Relationship of rumen fluid dilution rate to rumen fermentation and dietary characteristics of beef steers. J. Anim. Sci. 60:1061.

Felix, A., R. M. Cook and J. R. Huber. 1980. Effect of feeding isoacids with urea on growth and nutrient utilization by lactating cows. J. Dairy Sci. 63:840.

Ferrell, C. L., J. D. Crouse and R. A. Field. 1979. Effect of sex, diet and stage of growth upon energy utilization by lambs. J. Anim. Sci. 49:790.

Ferrell, C. L. and T. G. Jenkins. 1985. Cow type and the nutritional environment: nutritional aspects. J. Anim. Sci. 61:725.

Galyean, M. L. and F. R. McCollum. 1985. Influence of cottonseed meal supplementation on voluntary intake, rumen fermentation and rate of passage of prairie hay in beef steers. J. Anim. Sci. 60:570.

Gill, J. W. and K. W. King. 1958. Nutritional characteristics of Butyrivibrio. J. Bacteriol. 75:666.

Gorosito, A. R., J. B. Russell and P.J. Van Soest. 1985. Effect of carbon-4 and carbon-5 fatty acids on digestion of plant cell wall in vitro. J. Dairy Sci. 68:840.

Goetsch, A. L. and M. L. Galyean. 1982. Effect of dietary concentration level on rumen fluid dilution rate. Can. J. Anim. Sci. 62:727.

Grovum, W. L. and V. J. Williams. 1977. Rate of passage of digesta in sheep. 6. The effect of level of food intake on predictions of the kinetics of digesta in the reticulo-rumen and intestines. Brit. J. Nutr. 38:425.

Harrison, D. G., D. E. Beever, D. J. Thomson and D. F. Osvourn. 1975. Manipulation of rumen fermentation in sheep by increasing the rate of flow of water from the rumen. J. Agr. Sci. 85:93.

Hemsley, J. A. and R. J. Moir. 1963. The influence of higher volatile fatty acids on the intake of urea-supplemented low quality cereal hay by sheep. Aust. J. Agric. Res. 14:509.

Hespell, R. B. and M. P. Bryant. 1979. Efficiency of rumen microbial growth: influence of some theoretical and experimental factors on Y_{ATP}. J. Anim. Sci. 49:1640.

Hoover, W. H., R. J. Crawford, Jr. and M. D. Stein. 1982. Effects of solids and liquid flows on fermentation in continuous cultures. III. Solids retention time. J. Anim. Sci. 54:849.

Hume, I. D., R. J. Moir and M. Somers. 1970. Synthesis of microbial protein in the rumen I. Influence of the level of nitrogen intake. Aust. J. Agr. Res. 21:283.

Hungate, R. E. 1966. The Rumen and its Microbes, Academic Press, London and new York.

Kang-Meznarich, J. H. and G. A. Broderick. 1981. Effects of incremental urea supplementation on ruminal ammonia concentration and bacterial protein formation. J. Anim. Sci. 50:422.

Maeng, W. J., C. J. Van Nevel, R. L. Baldwin, and J. G. Morris. 1976. Rumen microbial growth rates and yields: effect of amino acids and protein. J. Dairy Sci. 59:68.

Maeng, W. J., and R. L. Baldwin. 1976a. Factors influencing rumen microbial growth rates and yields: effect of urea and amino acids over time. J. Dairy Sci. 59:643.

Maeng, W. J., and R. L. Baldwin. 1976b. Factors influencing rumen microbial growth rates and yields: effect of amino acid additions to a purified diet with nitrogen from urea. J. Dairy Sci. 59:648.

McDougall, E. L. 1984. Studies on ruminant saliva. I. The composition and output of sheep's saliva. Biochem. J. 43:99.

Mehrez, A. Z., R. E. Orskov and I. McDonald. 1977. Rates of rumen fermentation in relation to ammonia concentration. Br. J. Nutr. 38:437.

Mertens, D. R. 1977. Dietary fiber components: relationship to the rate and extent of ruminal digestion. Fed. Proc. 36:187.

Mertens, D. R. and L. O. Ely. 1979. A dynamic model of fiber digestion and passage in the ruminant for evaluating forage quality. J. Anim. Sci. 49:1085.

Mertens, D. R. and L. O. Ely. 1982. Relationship of rate and extent of digestion to forage utilization - a dynamic model evaluation. J. Anim. Sci. 54:895.

Mertens, D. R. and J. R. Loften. 1980. The effects of starch on forage fiber digestion kinetics in vitro. J. Dairy Sci. 63:1437.

Miura, H., M. Horiguchi, and T. Matsumoto. 1980. Nutritional interdependence among rumen bacteria, Bacteroides amylophilus, Megaspaera elsdenii, and Ruminococcus albus. Appl. Environ. Microbiol. 40:294.

Miura, H., M. Horiguchi, K. Ogimoto, and T. Matsumoto. 1983. Nutritional interdependence among rumen bacteria during cellulose digestion in vitro. Appl. Environ. Microbiol. 45:726.

Moore, L. A. 1964. Symposium on forage utilization: nutritive value of forage as affected by physical form. Part I. General principles involved with ruminants and effect of feeding pelleted or wafered forage to dairy cattle. J. Anim. Sci. 23:230.

Murphy, M. R., and J. M. Nicoletti. 1984. Potential reduction of forage and rumen digesta particle size by microbial action. J. Dairy Sci. 67:1221.

National Research Council. 1976. Nutrient requirements of domestic animals. Nutrient requirement of beef cattle. 5th rev. ed. National Academy of Sciences, Washington, D.C.

Nolan, J. V., and R. A. Leng. 1972. Dynamic aspects of ammonia and urea metabolism in sheep. Br. J. Nutr. 24:589.

Owens, T. N. and H. R. Isaacson. 1977. Ruminal microbial yields: Factors influencing synthesis and bypass. Fed. Proc. 36:198.

Pearce, G. R. 1967. Changes in particle size in the reticulorumen of sheep. Aust. J. Agr. Res. 18:119.

Pearce, G.R. and R.J. Moir. 1964. Rumination in sheep. Part I. The influence of rumination and grinding upon passage and digestion of food. Aust. J. Agr. Res. 15:635.

Prigge, E.C., M.J. Baker and F.A. Varga. 1984. Comparative digestion, rumen fermentation and kinetics of forage diets by steers and wethers. J. Anim. Sci. 59:237.

Pritchard, R. H. and J. R. Males. 1982. Effect of supplementation of wheat straw diets twice a day on rumen ammonia, volatile fatty acids and cow performance. J. Anim. Sci. 54:1243.

Rogers, J. A., B. C. Marks, C. L. Davis, and J. H. Clark. 1979. Alteration of rumen fermentation in steers by increasing rumen fluid dilution rate with mineral salts. J. Dairy Sci. 62:1599.

Russell, J. B., W. M. Sharp and R. L. Baldwin. 1979. The effect of pH on maximum bacterial growth rate and its possible role as a determinant of bacterial competition in the rumen. J. Anim. Sci. 48:251.

Russell, J. B. and C. J. Sniffen. 1984. Effect of carbon-4 and carbon-5 volatile fatty acids on growth of mixed rumen bacteria in vitro. J. Dairy Sci. 67:987.

Salter, D. N., K. Daneshaver and R. H. Smith. 1979. The origin of nitrogen incorporated into compounds in the rumen bacteria of steers given protein- and urea-containing diets. Brit. J. Nutr. 41:197.

SAS. 1985. Statistical Analysis System. SAS Institute Inc. Raleigh, North Carolina.

Satter, L. D. and L. L. Slyter. 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. Brit. J. Nutr. 32:199.

Simpson, M. E. 1984. Protective effect of alternative carbon sources toward cellulose during its digestion in bovine ruminal fluid. Developments in Industrial Microbiology. 25:641.

Slyter, L. L., and J. M. Weaver. 1971. Growth factor requirements of ruminal cellulolytic bacteria isolated from microbial population supplied diets with or without rapidly fermentable carbohydrate. Appl. Environ. Microbiol. 22:930.

Smith, L. W., H. K. Goering and C. H. Gordon. 1972. Relationships of forage compositions with digestion rates of cell wall components. J. Dairy Sci. 55:1140.

Smith, L. W., B. T. Weinland and D. R. Waldo. 1983. Rate of plant cell wall particle size reduction in the rumen. J. Dairy Sci. 66:2124.

Smith, W. R., I. Yu and R. E. Hungate. 1973. Factors affecting cellulolysis by Ruminococcus albus. J. Bacteriol. 114:729.

Snedecor, C. W. and W. G. Cochran. 1980. Statistical Methods, 7th Edition. Iowa State Press.

Soofi, R., G. C. Fahey, Jr., L. L. Berger and F. C. Hinds. 1982. Effect of branched chain volatile fatty acids, trypticase, urea and starch on in vitro dry matter disappearance of soybean stover. J. Dairy Sci. 65:1748.

Stewart, C. S. 1977. Factors affecting the celluloytic activity of rumen contents. Appl. Environ. Microbiol. 33:497.

Thorton, R. F. and D. J. Minson. 1972. The relationship between voluntary intake and mean apparent retention time in the rumen. Australian J. Agr. Res. 23:871.

Uden, P., P. E. Colucci and P. J. Van Soest. 1980. Investigation of chromium, cerium and cobalt as markers in digesta rate of passage studies. J. Sci. Food Agr. 40:337.

Uden, P. and P. J. Van Soest. 1984. Investigations of the in situ bag technique and a comparison of the fermentation in heifers, sheep, ponies and rabbits. J. Anim. Sci. 58:213.

Van Soest, P. F., and J. B. Robertson. 1980. Systems of analysis of evaluating fibrous feeds. p. 49-60. In W.J. Pigden, C.C. Balch, Michael Graham (ed.) Standardization ofanalytical methodology for feeds. IRDC Publ. No. 134e. Ottawa, Canada.

Varga, G. A. and W. H. Hoover. 1983. Rate and extent of neutral detergent fiber degradation of feedstuffs in situ. J. Dairy Sci. 66:2109.

Waldo, D. R. and L. W. Smith. 1972. Model of cellulose disappearance from the rumen. J. Dairy Sci. 55:125.

Welch, J. G. 1982. Rumination, particle size and passage from the rumen. J. Anim. Sci. 54:885.

Williams, C. H., D. J. David and O. Iismaa. 1962. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. J. Agric. Sci. 59:381.

Wohlt, J. E., J. H. Clark, and F. S. Blaisdell. 1976. Effect of sampling location, time and method of determining concentration of ammonia nitrogen in rumen fluid. J. Dairy Sci. 59:459.

Wolin, M. J. 1979. The rumen fermentation: a model for microbial interactions in anaerobic ecosystems. Advances in Microbial Ecology. 3:49.

Wolin, M. J. and T. L. Miller. 1983. Interactions of microbial populations in cellulose fermentation. Fed. Proc. 42:109.

APPENDIX

Table 1. Composition of the supplements[a] evaluated in the in situ trial and the heifer growth trial

Ingredients %	IFN[b]	DL-methionine-urea	SBM[c]	Urea	SBM-urea
Beet Pulp	4-00-669	93.22	66.68	92.72	85.37
DL-Methionine		1.14	--	--	--
Urea	5-05-070	3.68	--	3.40	2.85
Soybean Meal	5-04-612	--	31.00	--	9.82
Molasses	4-00-669	1.94	2.30	1.93	1.94
Vitamin A & D[d]		.02	.02	.02	.02
Ammonium Sulfate	6-09-339	--	--	1.93	--
Crude Protein, as fed basis		10.67	9.93	10.10	9.93

[a]The SBM-urea supplement was used in the heifer growth trial only.

[b]International Feed Number

[c]Soybean Meal

[d]Vitamin A & D premix contained 44,000,000 IU/kg vitamin A and 8,800,00 IU/kg vitamin D.

Table 2. Influence of in vitro buffer amendments on dry matter (DM) disappearance and fermentation rate of native winter range forage esophageal extrusa

Amendment	DM disappearance[a] %	Fermentation rate[bc] %/hour
None	41.2^{def}	2.7^{de}
Arginine	47.1^{d}	3.6^{i}
Cysteine	44.8^{de}	3.4^{fghi}
Histidine	46.3^{de}	3.5^{hi}
Leucine	44.6^{de}	3.4^{ghi}
Lysine	37.1^{f}	2.6^{d}
Methionine	47.4^{d}	3.6^{hi}
Tryptophan	43.2^{def}	3.1^{defghi}
Phenylalanine	39.9^{ef}	2.8^{def}
Isoleucine	45.8^{de}	3.3^{efghi}
Isobutyric acid	36.9^{f}	2.9^{defg}
Isovaleric acid	43.3^{def}	3.0^{defgh}
2-Methylbutyric acid	44.3^{de}	3.1^{defghi}

[a]Average for both runs and standard error ± 2.1.

[b]Average for both runs and standard error ± 0.2.

[c]$Dig = ae^{bx}$, whereas b = rate of digestion and are the values listed above.

^{defghi}Means within columns with different superscripts are different ($P<.05$).

Table 3. Influence of in vitro buffer amendments on neutral detergent fiber (NDF) disappearance and fermentation rate of native winter range forage esophageal extrusa

Amendment	NDF disappearance[a] %	Fermentation rate[b] %/hour
Forage only	53.1^{cde}	2.5^{c}
Arginine	56.9^{cd}	3.0^{cde}
Cysteine	53.2^{cde}	3.9^{f}
Histidine	57.8^{c}	2.9^{cde}
Leucine	54.5^{cd}	3.4^{de}
Lysine	51.5^{cde}	2.8^{cd}
Methionine	55.8^{cd}	3.8^{de}
Tryptophan	51.1^{de}	3.0^{cde}
Phenylalanine	47.9^{e}	2.7^{c}
Isoleucine	56.6^{cd}	2.9^{cde}
Isobutyric acid	50.9^{de}	2.9^{cde}
Isovaleric acid	56.2^{cd}	2.7^{c}
2-Methylbutyric acid	56.6^{cd}	2.9^{cd}

[a]Average for both runs and standard error ± 2.2.

[b]Average for both runs and standard error ± 0.2.

^{cdef}Means within columns with different superscripts are different ($P<.05$).

Table 4. Influence of in vitro buffer amendments on dry matter (DM) and neutral detergent fiber (NDF) fermentation lag time of native winter range forage esophageal extrusa

Amendment	DM lag time[a] hours	NDF lag time[b] hours
Forage only	28.0[c]	21.9[c]
Arginine	30.3[c]	24.6[c]
Cysteine	29.9[c]	25.0[c]
Histidine	30.2[c]	23.5[c]
Leucine	29.9[c]	25.1[c]
Lysine	30.2[c]	20.2[c]
Methionine	29.9[c]	25.2[c]
Tryptophan	29.8[c]	25.1[c]
Phenylalanine	29.5[c]	24.3[c]
Isoleucine	29.4[c]	22.5[c]
Isobutyric acid	32.1[c]	23.4[c]
Isovaleric acid	29.1[c]	20.3[c]
2-Methylbutyric acid	28.8[c]	22.4[c]

[a]Average for both runs and standard error ± 2.1.

[b]Average for both runs and standard error ± 2.3.

[c]Means within columns with different superscripts are different ($P<.05$).

Table 5. In situ dry matter fermentation rate, particulate passage rate and rumen digestibility for forage[a] as influenced by supplement treatments

Measurements	Supplements			
	DL-methionine-urea	Soybean Meal	Urea	SE[b]
Fermentation rate, %/h	9.53[c]	7.28[d]	7.74[d]	.071
Particulate passage rate, %/h	4.20[c]	4.92[d]	4.24[c]	.026
Rumen Digestibility, % (calculated)	48.49[e]	42.40[f]	47.55[e]	2.37

[a]A low quality forage consisting of 75% mature grass hay and 25% barley straw.

[b]± Standard error of the mean.

[cd]Means within the same row with different superscripts are different ($P<.05$).

[ef]Means within the same row with different superscripts are different ($P<.10$).

Table 6. In situ neutral detergent fiber fermentation rate, particulate passage rate, rumen digestibility and fermentation lag time for forage[a] as influenced by supplement treatments

Measurements	Supplements			
	DL-methionine-urea	Soybean Meal	Urea	SE[b]
Fermentation rate, %/h	5.13	4.01	4.23	.05
Particulate passage rate, %/h	4.20[c]	4.92[d]	4.24[c]	.03
Rumen Digestibility, % (calculated)	39.21	36.53	35.28	3.42
Fermentation lag time, h	1.09	1.05	1.77	.41

[a]A low quality forage consisting of 75% mature grass hay and 25% barley straw.

[b]± Standard error of the mean.

[cd]Means within the same row with different superscripts are different ($P<.05$).

Table 7. Rumen liquid parameters as influenced by supplement treatments during the in situ trial

Rumen measurements	Supplements			
	DL-methionine-urea	Soybean Meal	Urea	SE[a]
Fluid volume, liters	40.91	41.75	41.86	.41
Fluid dilution rate, %/h	10.62[b]	9.77[c]	10.53[b]	.02
Fluid outflow, liters/h	3.89	4.28	4.00	.23
Fluid turnover time, h	9.50[b]	10.25[c]	9.56[b]	.18

[a] ± Standard error of the mean.

[bc] Means within the same row with different supercripts are different (P<.01).

Table 8. Rumen ammonia levels[a] as influenced by supplement treatments in the in situ trial

Collection hours	Supplements			
	DL-methionine-urea	Soybean Meal	Urea	SEb
4	26.38[c]	11.01[d]	25.72[c]	5.72
8	8.30	4.93	9.32	4.18
12	5.01	4.25	3.73	.68
18	4.90	5.25	4.16	1.39
24	23.53	9.18	8.15	6.25
36	3.81	5.34	4.51	1.61
48	8.72	9.84	9.04	1.91
72	8.58	10.46	9.44	1.03
96	6.24	7.97	5.35	2.07
Entire trial	9.53	8.26	9.22	3.96

[a]Ammonia levels are reported as mg/100 ml rumen fluid.

[b]±Standard error of the mean.

[cd]Means within the same row with different superscripts are different ($P<.05$).

Table 9. Average daily gain, feed intake, and feed efficiency of heifer calves as influenced by supplements

Measurements	Supplements				
	DL-methionine-urea	SBM[a]	Urea	SBM-urea	SE[b]
Average Daily Gain, kg/(head day^{-1})	.60	.64	.62	.60	.02
Feed Intake, kg/(head day^{-1})	6.83	6.74	6.74	7.29	.28
Feed Efficiency, kg dm/kg gain	11.44	10.71	11.75	11.26	.55

[a]Soybean Meal

[b]±Standard error of the mean

DATE	ISSUED TO